42. (NEW) The method of claim 41, wherein the mammalian cell is a human cell or a mouse cell.

43. (NEW) The method of claim 38, wherein the cell is a mammalian cell.

44. (NEW) The method of claim 43, wherein the mammalian cell is a human cell.

REMARKS

I. Introduction

In response to the Office Action dated October 3, 2001, claims 17, 20-30, 35 and 37 have been amended, and new claims 41-44 have been added. Claims 17-44 remain in the application. The specification has been amended to correct obvious typographical errors. Reconsideration of the application, and entry of the amendments, is respectfully requested.

II. Claim Amendments

Applicants' attorney has made amendments to the claims as indicated above. These amendments were made solely for the purpose of clarifying the language of the claims, and were not required for patentability or to distinguish the claims over the prior art. These amendments are supported by the application as originally filed, and introduce no new matter.

Claims 17 and 36 have been amended to clarify that the phrase "consisting essentially of" means that the replacement DNA fragment does not include vector sequence, nor does it include all exons of a gene. This language is supported by the application as filed, for example, at page 77, lines 13-31, and at page 7, lines 3-10. In addition, the recitation of "intronic" has been replaced with "noncoding" for clarification and to be consistent with the terminology used throughout the specification, such as at page 22, lines 4-7.

The amendment to the last line of claim 21 is supported by the specification at page 21, lines 5-6.

The amendment to claims 23 and 35 is supported by the specification at page 51, line 9.

The amendment to claim 28 is supported by the specification at page 73, lines 8-10.

New claims 41-44 are supported by the specification at page 55, lines 27-28, and at page 58, line 19.

III. Examiner Interview Summary

Record is made of an interview on October 19, 2001, between Applicants' undersigned attorney, and Examiners Loeb and Schwartzman in connection with the present patent application. The discussion centered on clarification of the inventive concept underlying the claimed subject matter, and the meaning of the recitation of "consisting essentially of" in the independent claims as they relate to the inventive concept. Additional discussion related to the rejections based on 35 USC §112, first and second paragraphs, and the sufficiency of proposed amendments and evidence to overcome these rejections.

Applicants gratefully acknowledge and appreciate the helpfulness and suggestions provided by both Examiners Loeb and Schwartzman during this interview. The amendments, arguments and evidence presented herein are based on careful consideration of the contents of the interview, the specification and the cited references, and a good faith belief to be consistent therewith. Should the Examiner believe that this consistency is not present or is not sufficient to obtain allowance of the claims, the courtesy of a telephone call to Applicants' undersigned representative would be greatly appreciated.

IV. Double Patenting Rejection

At pages 2-3 of the Office Action, claims 17, 20-26 and 28-36 were rejected under the judicially created doctrine of obviousness-type double-patenting in view of claims 1-10 and 12 of prior U.S. Patent No. 6,010,908. Applicants will provide a terminal disclaimer upon identification of allowable subject matter.

V. Claim Objection

At page 3 of the Office Action, the Examiner objected to claim 21 because of the misspelling of "cell". Claim 21 has been amended to correct this error.

VI. Rejections Under 35 USC §112

A. Rejections Under 35 USC §112, First Paragraph

At pages 3-6 of the Office Action, claims 18, 19 and 38-40 were rejected under 35 USC §112, first paragraph, as containing subject matter not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. At pages 6-7 of the Office Action, claims 17 and 20-36 were rejected under 35 USC §112, first paragraph, as containing subject matter not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Paragraph 6 at page 6 of the Office Action, states that the specification is enabling for a method for replacing a target fragment in a cell *in vitro*, but does not reasonably provide enablement for a method of replacing a target fragment *in vivo* or *ex vivo*, wherein the cells are intended for gene therapy use. Applicants respectfully traverse these rejections for the following reasons, and request that the rejections be withdrawn.

1. Gene therapy is not *per se* an unpredictable art.

At page 4 of the Office Action, the Examiner asserts that the state of the art of gene therapy, at the time the invention was made, shows a "complete lack of documented success for any treatment based on gene therapy". The Examiner cites the Verma and Palu references as teaching that "no successful outcome has been achieved". These statements must be viewed, however, in the context of the references as a whole. Moreover, if the assertion that no gene therapy has ever been successful is taken as a basis for demonstrating a lack of enablement, it would imply that the Patent Office does not consider any methods of gene therapy to be patentable subject matter.

Both the Verma and Palu references acknowledge that gene therapy has resulted in gene transfer and expression (see Verma at p. 242, col. 1, and Palu Abstract). Verma et al. concludes by acknowledging that the promises of gene therapy are great and the problems surmountable (p. 242, final paragraph). The thrust of these articles is an evaluation of the obstacles to be overcome before gene therapy becomes a routine clinical practice. The Verma and Palu references are not reviewing the results of early gene therapy trials with the standard for patentability in mind. Rather, these authors are addressing what is needed for gene therapy to meet a standard for statistically significant proof of efficacy in treating disease sufficient to warrant undertaking the risks of applying these

methods to humans on a routine basis. Even with that concern in mind, it must be acknowledged that over 300 clinical trials would not be approved and undertaken if those skilled in the art did not have a reasonable expectation of success with gene therapy.

Moreover, other publications reporting results of the first human gene therapy clinical trial (see, e.g. NHGRI Press Release: Results From First Human Gene Therapy Clinical Trial, available at http://www.nhgri.nih.gov/Policy_and_public_affairs/Communications/Press_releases/clinical_trial.html, a copy of which is attached as Exhibit A) state that the "results indicate replacement genes can be expressed stably in white blood cells over long periods of time." Applicants maintain that stable expression of transferred or altered genetic material *in vivo* is one example of an application of gene therapy that is regarded as successful by those skilled in the art. Further evidence that those skilled in the art have a reasonable expectation of success using Applicants' small fragment homologous replacement method and other more recently developed gene therapy strategies is provided by an article by Ferber that appeared in the November 23, 2001, issue of *Science* (Volume 294, pages 1638-1642). A copy of Ferber's article is attached herewith as Exhibit B.

2. Applicants have met the legal standard for enablement.

The Examiner is respectfully reminded that it is not the Patent Office's duty to ensure that a method is successful in some minimal percentage of cases prior to allowing grant of a patent. If the method is enabled for replacing target gene fragments in even a small percentage of instances, the method is enabled. Improvements in efficiency of delivery and sustained expression, a concern raised at page 4 of the Office Action, are precisely what is addressed by Applicants' disclosure. As disclosed in the specification, the flanking noncoding sequences and absence of vector sequence facilitate efficient fragment replacement.

The legal standard for enablement requires the Examiner to provide a reasonable basis to question the enablement provided in the disclosure for the claimed invention (MPEP §2164.04). While predictability of the general field of technology is relevant to an enablement analysis, the critical issue is whether the disclosure of the specification and knowledge in the art are sufficient to enable the particular subject matter claimed. More specifically, if the examples and teachings of the specification are predictive of success with the claimed invention, the fact that other gene therapy and gene replacement methods are less efficient does not negate the showing of enablement.

The citations from Verma and Palu are not directed to use of small fragment homologous replacement, they are instead directed to the general limitations of treating human disease with gene therapy and deficiencies in transgene expression. Moreover, these references do not state that no transgene expression is achieved. As discussed at page 19, lines 9-19, of the specification, more than 500 mutations of the CFTR gene are known to give rise to cystic fibrosis, the most common of which is the ΔF508 deletion. Genetic corrections to address other diseases are discussed in the ensuing paragraphs at pages 19-21 of the specification. Applicants' invention addresses the need for such corrections in a therapeutically feasible manner by providing for an efficient and effective method for obtaining expression of a replacement DNA fragment.

The data disclosed in the specification at pages 25-54 demonstrate that small fragment homologous replacement, in accordance with the method of claim 17, successfully targets and modifies genetic sequences associated with cystic fibrosis and sickle cell anemia. In the examples concerning cystic fibrosis, several different cell lines were used and shown to successfully contain the correct sequence at the appropriate genomic locus, to express mRNA containing the corrected sequence, and to exhibit functional correction as demonstrated by reversal of the ion transport defect associated with cystic fibrosis. The Office Action presents no reasoning or evidence to question that these successful modifications demonstrated *in vitro* are not predictive of successful *in vivo* modification.

MPEP §2164.03 states that the "'predictability or lack thereof' in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention." The subject invention involves a model system that differs from the general field of gene therapy referred to in the Verma and Palu references. The arguments raised in the Office Action with respect to unpredictability in the art of gene therapy are not relevant to the predictability of specific correction of known genetic defects using small fragment homologous replacement. Even the Luo et al. reference cited in the Office Action is similarly directed to a broader category of gene therapy and does acknowledge that some successes have been achieved (page 33, column 2, line 1). Moreover, Luo et al. recognizes the advances achievable via optimization of such factors as size of DNA complexes and targeting, strategies that are addressed by Applicants' disclosure.

3. Enablement of the claimed method has been confirmed with *in vivo* and *ex vivo* data.

Confirmation that the successes described in Applicants' specification using *in vitro* and *ex vivo* model systems are indeed predictive of successful application of the claimed method to the *in vivo* environment is demonstrated by recent publications by the inventors and others. Goncz et al., 2001, Gene Therapy 8:961-965 (Exhibit C), demonstrates successful replacement and *in vivo* expression of a ΔF508 fragment in the lung of normal mice using the method of claim 17 and following the teachings of the specification. Kunzelmann et al., 1996, Gene Therapy 3:859-867 (Exhibit D), shows that correction of the ΔF508 mutation with a wild type fragment, in accordance with the method of claim 17, results in replacement at the appropriate genomic locus, mRNA expression of the replacement fragment and restoration of chloride transport, thereby correcting the functional defect that gives rise to the cystic fibrosis phenotype. Goncz et al., 1998, Human Molecular Genetics 7(12):1913-1919 (Exhibit E), demonstrates that the successful fragment replacement demonstrated by Kunzelmann et al. (Exhibit D) is not limited to transformed cells, but also occurs in non-transformed primary human airway epithelial cells. It is these successes that are acknowledged in the recent Science article attached as Exhibit B (see box entitled "Repair Kits for Faulty Genes" at top of page 1639).

Kapsa et al., 2001, Human Gene Therapy 12:629-642 (Exhibit F), demonstrates successful *in vivo* correction of the *mdx* dystrophin gene nonsense mutation in a mouse model of Duchenne muscular dystrophy, also using the method of Applicants' claim 17 and following the teachings of the specification. In yet another model system, *ex vivo* replacement using Applicants' method and a fragment containing the β^S-globin mutation associated with sickle cell anemia was successfully demonstrated in human hematopoietic stem/progenitor cells as well as human bronchial epithelial cells. The small fragment homologous replacement was stable for at least 5 weeks, and occurred in up to 70% of alleles in the outgrowth population of cells. These data are described in a Goncz et al. abstract (Exhibit G) of a presentation made at a September 24, 2001, Working Group Meeting on "Technologies for *in situ* Repair of Single Nucleotide Mutations in Single Gene-Defect Blood Diseases", sponsored by The National Heart, Lung, and Blood Institute of the National Institutes of Health.

In view of the arguments and evidence of successful replacement of DNA fragments using the claimed methods and of functional correction that is predictive of therapeutic treatment presented herein, Applicants respectfully maintain that the teachings of the specification provide adequate support to enable those skilled in the art to make and use the subject matter of claims 17-44. Withdrawal of these rejections under 35 USC §112, first paragraph, is therefore respectfully requested.

B. Rejections Under 35 USC §112, Second Paragraph

At pages 7-9 of the Office Action, claims 20-36 were rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants amendments to the claims, by clarifying the recited terms and correcting typographical errors, obviate these rejections. Withdrawal of these rejections under 35 USC §112, second paragraph, is therefore respectfully requested.

VII. Rejections Based on Prior Art

A. Rejections Under 35 USC §102

At page 9 of the Office Action, the Examiner asserts that the phrase "consisting essentially of" with respect to DNA has been interpreted as open language in the absence of a definition in the specification, and further that additional sequence is encompassed by such claims as long as such additional sequence does not change the basic nature of the invention. Applicants agree with the second statement, but respectfully point out that the first statement is contrary to legal precedent as discussed in MPEP §2111.03. "Consisting essentially of" is not open-ended and limits the scope of the claim to the specified materials and those that do not materially affect the basic and novel characteristics of the claimed invention (see *Id.*, citing *in re Herz*, 190 USPQ 461, 463, CCPA 1976).

As discussed hereinabove, Applicants' specification makes it clear that the basic nature of the invention relates to the use of small replacement DNA fragments that encompass one or more replacement exons flanked by homologous noncoding sequence. It is this noncoding flanking sequence, and the absence of further material beyond the flanking noncoding sequence, that is disclosed as resulting in improved efficiency of target gene correction.

At page 10 of the Office Action, claims 17-20, 27-30, 32 and 37-40 were rejected under 35 USC §102(e) as allegedly anticipated by Berns et al. (US Patent No. 5,789,215). As acknowledged by

the Examiner, Berns teaches use of a plasmid vector containing two exons of interest. This plasmid sequence constitutes vector sequence, which is excluded by Applicants' claims.

At pages 10-11 of the Office Action, claims 17-20, 26-29, 31 and 37-40 were rejected under 35 USC §102(b) as allegedly anticipated by Vega (Human Genetics, 1991, 87:245-253). Vega is a review article that discusses three methods of gene therapy (see last paragraph at page 245), each of which involves introduction of foreign genes and/or vector sequence, elements excluded by Applicants' claims.

At pages 11-12 of the Office Action, claims 17, 20, 21, 23, 27-30, 32 and 37 were rejected under 35 USC §102(b) as allegedly anticipated by Kay et al. (US Patent No. 5,612,205). The teachings of Kay, however, involve the use of plasmids containing 50 kb pieces of DNA that include substantial nonhomologous regions, and therefore include elements excluded by Applicants' claims.

At page 12 of the Office Action, claim 37 was rejected under 35 USC §102(b) as allegedly anticipated by Tsui et al. (WO 91/10734). Tsui merely discloses a number of specific mutations that give rise to cystic fibrosis. Tsui does not teach or suggest how to correct such mutations in accordance with Applicants' claimed methods, nor does Tsui teach or suggest a composition comprising a replacement DNA fragment and a delivery vehicle suitable for delivery of the replacement DNA fragment into a cell. In addition, Tsui does not teach or suggest a replacement DNA fragment that consists essentially of (1) at least one replacement exon having a 3' end and a 5' end, (2) a 3' end consisting essentially of a 3' flanking noncoding sequence adjacent to the 3' end of the at least one replacement exon, and (3) a 5' end consisting essentially of a 5' flanking noncoding sequence adjacent to the 5' end of the at least one replacement exon, wherein the replacement DNA fragment includes less than all of the exons of the gene and does not include vector sequence, and wherein the 3' flanking noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 3' flanking noncoding sequence adjacent to the target fragment, and the 5' flanking noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 5' flanking noncoding sequence adjacent to the target fragment.

B. <u>Rejections Under 35 USC §103</u>

At page 13 of the Office Action, claims 17-20, 26-29, 31 and 36-40 were rejected under 35 USC §103(a) as allegedly unpatentable over Vega (Human Genetics, 1991, 87:245-253). As

discussed above, Vega is a review article that discusses three methods of gene therapy (see last paragraph at page 245), each of which involves introduction of foreign genes and/or vector sequence, elements excluded by Applicants' claims.

Accordingly, Applicants maintain that none of the cited references, either alone or in combination, teach or suggest the subject matter of claims 17-44. Prior to Applicants' disclosure, the advantages obtained by the use of small replacement DNA fragments that consist essentially of one or more replacement exons flanked by homologous noncoding sequence were not appreciated. Withdrawal of the rejections under 35 U.S.C. §§102-103 is therefore respectfully requested.

VIII. Conclusion

In view of the above, it is submitted that this application is now in good order for allowance and such allowance is respectfully solicited. Should the Examiner believe minor matters still remain that can be resolved in a telephone interview, the Examiner is urged to call Applicants' undersigned attorney.

Respectfully submitted,

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APPENDIX A: SPECIFICATION IN MARKED-UP FORM

Please replace the paragraphs at page 20, line 13, to page 21, line 24, with the following paragraphs:

Retinoblastoma (Rb) gene involves chromosomal abnormality on chromosome 13 q 14 (the long arm). Retinoblastoma is a malignant abnormality that results from the loss of the Rb gene. Although this gene is normally found in 2 copies (one on each chromosome 13), certain individuals will have one or both copies of the Rb gene deleted. If both copies of the gene are deleted, the onset of malignancy happens rapidly. If one copy is deleted, then a secondary mutation event is required before malignancy occurs. The gene product is involved with regulation of cell proliferation and its loss eliminates the tight controls placed on cell [diversion] division. In addition to retinoblastoma, a deleted or dysfunctional Rb gene has been associated with osteosarcomas and solid tumors in other organs.

There are numerous other genetic disorders and genes that lend themselves to the gene therapy protocols proposed in this invention. While the number of genes that could be altered by SFHR is large, a primary requirement will be knowledge of the DNA sequence that constitutes a given gene. When this information is known or obtained, the invention provides straight-forward method to design a SFHR gene therapy strategy that encompasses both the generation of the incoming therapeutic DNA and the analysis of the target cells after SFHR. In particular, inherited diseases that are the result of small mutation (e.g., base changes, small deletions or insertions) are particularly amenable to gene therapy by SFHR. In addition to those diseases mentioned above, SFHR gene therapy could be applied to adenosine deaminase deficiency (ADA), Lesch Nyhan syndrome, [Duchenes] Duchenne muscular dystrophy, and Fanconi's anemia, to mention a few. Furthermore, the SFHR gene therapy protocol could be applied to the treatment of certain infectious diseases.

In the treatment of infectious disease, the incoming exogenous DNA contains mutations that inactivate the pathogen by introducing these inactivating mutations into essential genes of the genome of the pathogen. Retroviral pathogens, such as HIV, the agents thought to be responsible for acquired immune deficiency syndrome (AIDS) and the [hepatitus] hepatitis B virus are candidates for SFHR gene therapy of infectious diseases. These viruses rely on the integration of the

proviral DNA into the cellular genome as part of their replicative cycle. The proviral DNA is the target for the incoming exogenous DNA fragments. A cocktail of fragments with inactivating mutations, that are homologous to several different essential genes within the viral genome is used to disrupt the integrity of the viral genome and the viruses ability to replicate.

Please replace the paragraph at page 24, lines 25-29, with the following paragraph:

The normal DNA fragment whether uncoated, coated or complexed is preferably delivered into the cell complexed with a protein-lipid complex[ed], complexed with a lipid layer or with a dendrimer or by electroporation or microinjection according to Examples 15-17.

Please replace the paragraph at page 25, lines 19-23, with the following paragraph:

Homologous replacement according to the invention has been tested, successfully achieved and a correction of a [disfunction] dysfunction in cystic fibrosis has been observed. Other diseases subject to the same treatment are sickle cell anemia and xeroderma pigmentosum group G.

Please replace the paragraph at page 39, lines 10-19, with the following paragraph:

Results in this study demonstrate that (a) .DELTA.F508 CF epithelial cells undergo homologous replacement at the CFTR mutation with small fragments of wtCFTR DNA, resulting in a corrected genomic .DELTA.F508 locus; (b) rec A protein-coated and uncoated [ssDNS] ssDNA fragments can be used in the transfection of cultured human cells; (c) cystic fibrosis .DELTA.F508 mutations corrected in genomic DNA result in production of normal CFTR mRNA; and (d) CF cells corrected by homologous replacement display intact cAMP-dependent Cl transport.

Please replace the paragraph at page 40, line 26, to page 41, line 20, with the following paragraph:

The patch clamp analysis shows that within a population of transfected CF cells there appears to be a significant subpopulation cells in which cAMP-dependent Cl transport defect has

been corrected. In these experiments precautions were taken to reduce the contribution of K.sup.+ currents to the whole cell current by having CsCl in the pipette filling solution. The continuous recording of cell membrane potential kept the cells at their own membrane potential and reduced artifacts due to cell swelling or voltage clamping. The increase in whole cell current in 7 of 78 forskolin treated cells and the depolarization due to the reduction of the bath Cl in each of the 7 responding cells, clearly shows that the current increase in the whole cell was due to activation of a Cl conductance. The apparent homologous replacement frequency of 9% is a maximum frequency in that it assumes that each responding cell represents a single homologous replacement event. However, the possibility that in cases where multiple responding cells were detected (GS- and SD+, Table 2), each responding cell may have been derived from a single cell in which homologous replacement occurred can not be ruled out. If this was the case, the responding cells would, in effect, reflect 3 separate [honmologous] homologous replacement events and would indicate a frequency of ~4% (3 in 74 cells). These values show close agreement with the calculation of frequency from the densitometric analysis of homologous replacement frequency (3-7%). In any event, the degree of homologous replacement and phenotypic correction is well within the range of 6-10% that appears to be sufficient for conversion of a CF epithelial monolayer to one with normal cAMP-dependent Cl transport properties as reported in Nat. Genetics, 2:2 (1992).

Please replace the paragraphs at page 48, lines 13-28, with the following paragraphs:

DNA samples from the T6/20 plasmid (lanes 1, 4, and 7), nonCF lymphocytes (lanes 2, 5, and 8), and CF airway epithelial cells, ΣCFTE29o- (lanes 3, 6, and 9). No PCR products were detected when one primer based on the previously published sequence was used (lanes 1-3). Primers based on sequence data reported here were able to show PCR products from all DNA samples (lanes 4-9).

An expected 485-bp fragment was detected using primer CF39 and C16D in DNA samples isolated from non-CF human lymphocytes, ΣCFTE29o-, a trachea epithelial cell line derived from a CF patient homozygous for the ΔF508 mutation and from the plasmid T6/20 seen in FIG. 17. Similarly, PCR amplification with primers CF40/CF41, localized outside previously published sequence, was able to generate the expected 1057-bp fragment from the three DNA samples

indicated above as seen in FIG. 17.

Please replace the paragraph at page 54, line 34, to page 55, line 4, with the following paragraph:

The normal DNA fragment is delivered into the cells under conditions effective for homologous replacement to occur. The resulting genetically altered cells can then be delivered to the subject in need of gene correction. This ex vivo alteration of cells and their transplantation into an afflicted individual comprises another mode of delivery of the [exonous] exogenous DNA fragment, as opposed to direct introduction of the fragments in vivo.

Please replace the paragraph at page 67, lines 6-11, with the following paragraph:

The β-gal reporter system indicates correction by the appearance of blue cells following X-gal treatment. Only the cells with a functional [β-galatosidase] β-galactosidase gene are blue. The fraction of blue cells within the population of transfected cells is readily determined by cell counting.

Please replace the paragraph at page 70, lines 22-31, with the following paragraph:

In addition, the above compounds according to the invention and their pharmaceutically acceptable derivatives may be employed in combination with other therapeutic agents for the treatment of the indicated conditions. Examples of such further therapeutic agents include agents that are effective for treatment of associated conditions. However, other agents that contribute to the treatment of the disease and/or its symptoms, such as inhibitors of [neutrophyl] neutrophil function, retinoic acid, anti-inflammatory agents, adenosine agonists, and the like, are suitable.

Please replace the paragraph at page 71, line 33, to page 72, line 2, with the following paragraph:

The delivery of the normal DNA fragment into the cell may be conducted by a variety of techniques discussed above. These encompass providing the altering DNA fragment enveloped by a

lipid layer, complexed with a protein and a lipid or [adendrimer] a dendrimer. The conditions for contacting any of these compositions with the cells to be altered were described above.

Please replace the paragraph at page 74, line 12, to page 75, line 7, with the following paragraph:

The cell recognition element is a molecule capable of recognizing a component on the surface of a targeted cell, covalently linked with a DNA-associating moiety by conventional methods. Cell recognition components include antibodies to cell surface antigens, ligands for cell surface receptors including those involved in receptor-mediated endocytosis, peptide hormones, etc. Specific ligands contemplated by this invention include carbohydrate ligands such as galactose, mannose, mannosyl 5-phosphate, fucose, sialic groups, N-acetylglucosamine or combinations of these groups as complex carbohydrates such as those found on glycolipids of the blood groups or on various secreted proteins. Other ligands include folate, biotin, various peptides that can interact with cell surface or intracellular receptors such as the chemoattractants peptide N-formyl-met-leu-phe, SEQ. ID. No.: 79 peptides containing the arg-asp-glycine sequence SEQ. ID. No.: 80 or cys-ser-gly-arg-glu-asp-val-trp SEQ. ID. NO.: 82 peptides, peptides that contain a [cystine] cysteine residue or that interact with cell surface protein such as the human immunodeficiency virus GP-120, and peptides that interact with CD-4. Other ligands include antibodies or antibody fragments. The specificity of the antibodies can be directed against a variety of epitopes that can be expressed on cell surfaces including histocompatibility macromolecules, autoimmune antigens, viral, parasitic or bacterial proteins. Other protein ligands include hormones such as growth hormone and insulin or protein growth factors such as GM-CSF, G-CSF, erythropoietin, epidermal growth factor, basic and acidic fibroblast growth factor and the like. Other protein ligands would include various cytokines that work through cell surface receptors such as interleukin 2, interleukin 1, tumor necrosis factor and suitable peptide fragments from such macromolecules.

Please replace the paragraph at page 77, lines 13-31, with the following paragraph:

The direct administration of small ssDNA fragments[,] advances previous homologous replacement because it shows that homologous replacement with small genomic DNA fragments is

successful to correct naturally occurring genomic CFTR mutations in CF epithelial cells, in sickle cells and skin cells. In addition, SFHR has an advantage over vector based homologous recombination strategies reported before in Nature, 346:847 (1990) because intron sequences are not disrupted by selectable marker gene sequences. This eliminates the possibility of interference of marker gene transcription with that of the targeted gene. The SFHR approach [also presents] also presents an advantage over cDNA gene therapy strategies, because the corrected gene continues to be regulated by endogenous genomic enhancers and promoters rather than a heterologous enhancer and promoter in the vector. Thus, homologous replacement increases the probability that the corrected gene, whether CFTR or another gene, is expressed in the appropriate cells at the appropriate levels.

Please replace the paragraph at page 81, lines 20-32, with the following paragraph:

The cells were then washed with 2 changes of PN buffer for 3 min at room temperature and incubated with biotinylated anti-avidin (5 μg in PNM solution) at room temperature for 20 min under a coverslip. The slides were washed with two changes of PN buffer, 3 min. each, at room temperature and the cells stained with FITC-avidin (5 μg/ml in PNM solution) at room temperature for 20 min under a glass coverslip. The slides were again washed 2 times, 3 min each, with PN buffer at room temperature. [Prospidium] Propidium iodide (2 μg/ml) was then added and the slides were viewed by fluorescence microscopy. The number of copies of chromosome 7 per cell was determined by counting fluorescent dots per nucleus.

Please replace the paragraph at page 90, lines 15-26, with the following paragraph:

The 491 bp fragment was prepared as described, denatured by heating to 95°C. for 10 min and then rapidly cooled on ice. 5 μl of the DNA were then added to a buffer solution. The buffer contained 20 mM Tris acetate, 10 mM Mg acetate, 70 mM [potasium] potassium acetate, 1 mM dithiothreitol, and 100 μg/ml bovine serum albumin-fraction V, 0.5 mM ATP-γ-S followed by the addition of UvsX: 20 μl of a 1.4 mg/ml solution according to J. Biol. Chem., 261:6107-6118 (1985); UvsY: 36 μl of a 0.5 mg/ml solution J. Biol. Chem., 268:15096-15103(1990); and T4 G p32: 100 μl

of a 5.2 mg/ml solution according to J. Biol. Chem., 263:9427-9436(1987) to a final volume of 250 µl.

Please replace the paragraph at page 96, lines 6-18, with the following paragraph:

Electroporation experiments were performed using recombinase-coated 491-mer ssDNA as described above. Approximately 10^7 exponentially growing cells were suspended in 400 µl of recombinase coating buffer with 5 µg (5 µl) of recombinase-coated DNA. The cell suspension was preincubated on ice for 10 mins. and electroporated at 4°C. with 400 V and 400 µF in a BTX 300 [elecroporator] electroporator (BTX Corporation, San Diego, Calif.). After electroporation, cells were incubated on ice for an additional 10 mins., diluted in Eagle's minimal essential medium (MEM) supplemented as described above, then seeded in a T75 flask. Under these electroporation conditions, approximately 30-50% of the cells survive. Cells were cultured at 37°C. in a humidified CO_2 incubator for 5-7 days and then harvested for DNA and RNA.

APPENDIX B: CLAIMS IN MARKED-UP FORM

17. (AMENDED) A method for replacing a target fragment of a gene in a cell, the method comprising delivering to the cell an exogenous replacement DNA fragment, the replacement DNA fragment consisting essentially of:

(a) at least one replacement exon having a 3' end and a 5' end;

(b) a 3' end consisting essentially of a 3' flanking [intronic] noncoding sequence adjacent to the 3' end of the at least one replacement exon; and

(c) a 5' end consisting essentially of a 5' flanking [intronic] noncoding sequence adjacent to the 5' end of the at least one replacement exon;

wherein the replacement DNA fragment includes less than all of the exons of the gene and does not include vector sequence, and wherein the 3' flanking [intronic] noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 3' flanking [intronic] noncoding sequence adjacent to the target fragment, and the 5' flanking [intronic] noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 5' flanking [intronic] noncoding sequence adjacent to the target fragment, so that the exogenous replacement DNA fragment replaces the target fragment of the gene in the cell.

20. (AMENDED) The method of claim 17, wherein the target fragment of the gene in the cell comprises a DNA sequence comprising a genetic defect [that controls] associated with a disease or dysfunction.

21. (AMENDED) The method of claim 20, wherein the disease or dysfunction is Fanconi's anemia, cystic fibrosis, sickle [call] cell anemia, thalassaemias, retinitis pigmentosa, xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, Duchenne's muscular dystrophy, Lesch Nyhan syndrome, adenosine deaminase deficiency or Tay-Sachs disease.

22. (AMENDED) The method of claim 20, wherein the target fragment of [a] the gene is a DNA sequence present in the cystic fibrosis gene.

23. (AMENDED) The method of claim 20, wherein the target fragment of [a] the gene is a DNA sequence present in the sickle cell anemia gene and the target fragment is replaced with a replacement genomic DNA sequence encoding a region of β-globin.

24. (AMENDED) The method of claim 20, wherein the [targeted mutant DNA sequence] target fragment of the gene is a DNA sequence present in the gene causing thalassaemias, wherein the sequence is replaced with a replacement genomic DNA sequence in the thalassaemias causing genomic loci.

25. (AMENDED) The method of claim 20, wherein the [targeted mutant DNA sequence] target fragment of the gene is a DNA sequence present in a gene causing xeroderma pigmentosum.

26. (AMENDED) The method of claim 17, wherein the replacement DNA fragment is single stranded.

27. (AMENDED) The method of claim 17, wherein the replacement DNA fragment is double stranded.

28. (AMENDED) The method of claim 17, wherein the delivering of the exogenous replacement DNA fragment comprises delivery by electroporation, microinjection, complexing the exogenous replacement DNA fragment [onto] in a lipid layer, complexing the exogenous replacement DNA fragment in a cationic lipid, complexing the exogenous replacement DNA fragment in a dendrimer or conjugating the exogenous replacement DNA fragment to polylysine.

29. (AMENDED) The method of claim 17, wherein the method is [directed at] carried out in a population of cells containing the target fragment of the gene, and further comprising determining the extent of homologous replacement by [PCR] identification of cells within the population that have replaced the target fragment of [a] the gene with the exogenous replacement DNA fragment at a target genomic locus, wherein the identification comprises PCR or Southern hybridization.

30. (AMENDED) The method of claim 29, wherein the exogenous replacement DNA fragment is identified using primers of about 25 bases that are outside of regions of homology defined by the exogenous replacement DNA fragment, or primers that are allele-specific and differentiate between the target fragment of [a] the gene and the exogenous replacement DNA fragment, or by Southern hybridization with allele-specific oligonucleotide probes that differentiate between the target fragment of a gene and the exogenous replacement DNA fragment.

35. (AMENDED) The method of claim 33, wherein the target fragment of a gene is a DNA sequence present in the sickle cell anemia gene, the target fragment is replaced with a replacement genomic DNA sequence encoding a region of β-globin, and the primers are selected from the group consisting of primers SC1(+), SC2(-), SC3(+), SC4(-), SC5(+), SC6(-), SC-BA(-) and SC- BS(-).

37. (AMENDED) A composition comprising a replacement DNA fragment and a delivery vehicle suitable for delivery of the replacement DNA fragment into a cell containing a target fragment of a gene, wherein the replacement DNA fragment consists essentially of:

(a) at least one replacement exon having a 3' end and a 5' end;

(b) a 3' end consisting essentially of a 3' flanking [intronic] noncoding sequence adjacent to the 3' end of the at least one replacement exon; and

(c) a 5' end consisting essentially of a 5' flanking [intronic] noncoding sequence adjacent to the 5' end of the at least one replacement exon;

wherein the replacement DNA fragment includes less than all of the exons of the gene and does not include vector sequence, and wherein the 3' flanking [intronic] noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 3' flanking [intronic] noncoding sequence adjacent to the target fragment, and the 5' flanking [intronic] noncoding sequence of the replacement DNA fragment is homologous to and anneals to a 5' flanking [intronic] noncoding sequence adjacent to the target fragment, so that the exogenous replacement DNA fragment replaces the target fragment of the gene upon delivery of the replacement DNA fragment into the cell.